Streptomyces coelicolor: DNA methylation and differentiation

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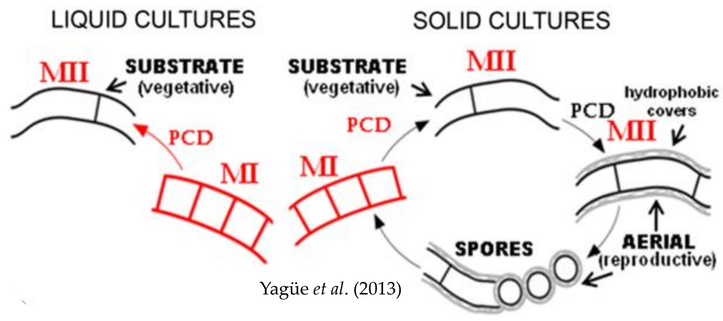
Abstract

DNA cytosine methylation is an epigenetic modification regulating many biological processes in eukaryotes, including chromatin organization, genome maintenance and gene expression. The role of DNA cytosine methylation in prokaryotes has not been deeply investigated.

In *Escherichia coli* it was recently demonstrated that cytosine methylation regulates gene expression during stationary phase [1] and that an induced state of cytosine hypermethylation leads to chromosomal DNA cleavage and cell death [2].

Streptomyces coelicolor is a mycelial soil microorganism, which exhibits a complex life cycle that includes three different cell types: unigenomic spores, a compartmentalized mycelium (MI) and a multinucleated mycelium (substrate and aerial mycelium, MII). This life cycle is finely regulated through several mechanisms (i.e. two events of programmed cell death, PCD) [3].



Yagüe *et al.* (2013)

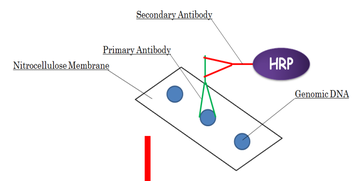
The importance of DNA methylation was already described in Streptomycetes [4], but its biological role remains unknown.

Aims

1. To analyze cytosine methylation pattern of *Streptomyces coelicolor* M145 during development on solid and in liquid culture.
2. To investigate the relationship between DNA cytosine methylation and morphological/physiological differentiation.

Methods

- Dot Blot analysis with Antibody against 5-methylcytosine

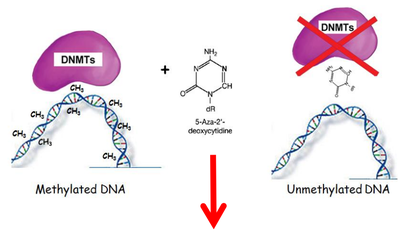


To check cytosine methylation of total genomic DNA

- Bisulfite sequencing

To analyze cytosine methylome

- Treatment with 5-aza-2'-deoxycytidine (aza-dC)

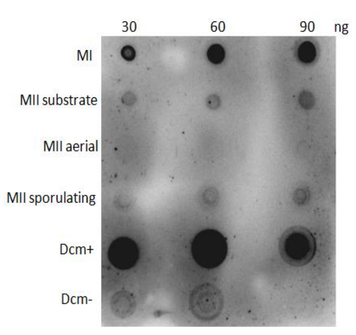


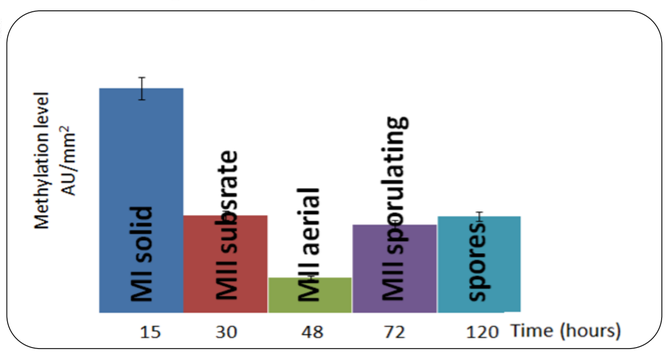
To investigate the biological effect of cytosine methylation

Results

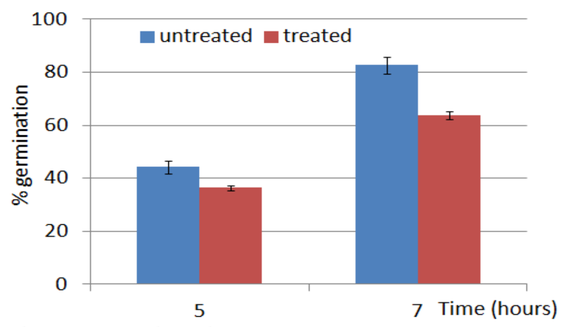
SOLID CULTURE

•Dot blot analysis revealed that the level of cytosine methylation changes during development in solid GYM. Specifically, DNA methylation is higher at the MI stage than in the MII (substrate, aerial and sporulating) and spores.

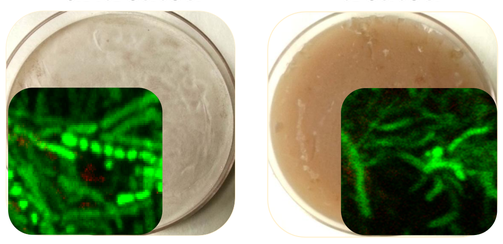




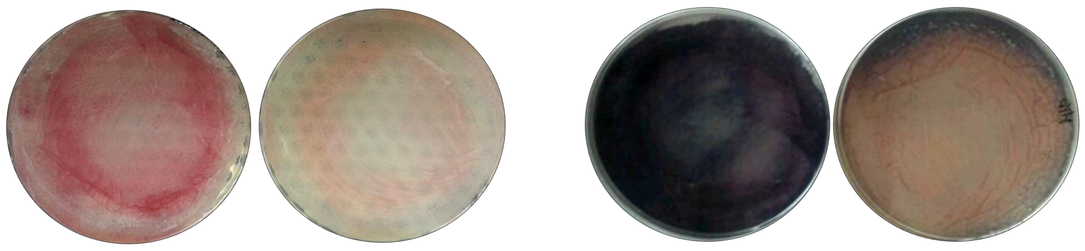
•Phenotypic analyses of cultures treated with aza-dC demonstrated that DNA methylation influences germination, sporulation and antibiotic production.



In the treated culture germination was decreased and delayed.



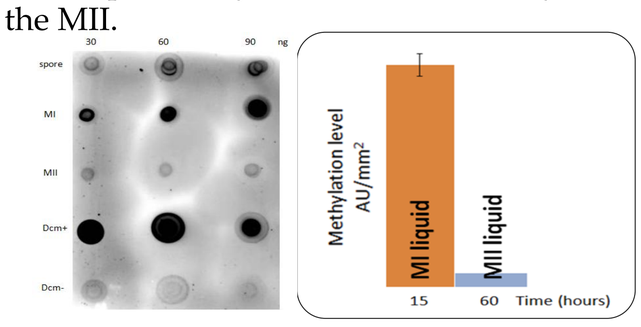
The sporulation (96h) was blocked in the treated culture.



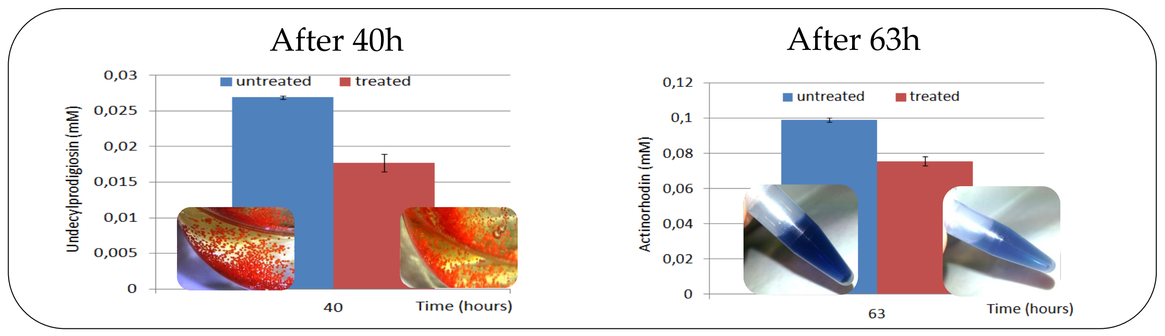
The undecylprodigiosin and actinorhodin production, characterized by the red and blue pigment, respectively, was delayed and decreased in the treated culture.

LIQUID CULTURE

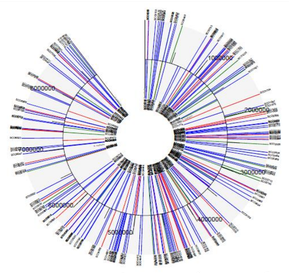
•The level of cytosine methylation changes during growth in liquid media, i.e. R5A, MG and JM. Specifically, in R5A DNA methylation is higher at the MI stage than in the MII.



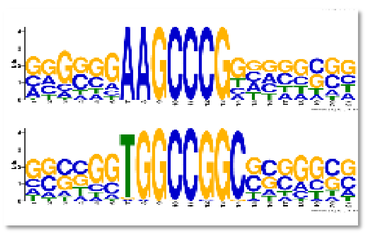
•Treatment of R5A culture with aza-dC resulted in a delayed and decreased production of undecylprodigiosin and actinorhodin.



•Preliminary analysis of bisulfite sequencing of MII phase of *S. coelicolor*, grown in liquid medium MG, revealed that 30% of genes contained a methylated motif in their upstream regions.



Representation of methylated cytosines in the genome of *S. coelicolor*



Methylated motifs found in the upstream region of ~2150 genes

Conclusions

1. The global level of cytosine methylation changes during development, in particular it is higher at the MI stage on solid and liquid medium.
2. DNA methylation influences antibiotic production both in liquid and on solid medium, and germination and sporulation on solid medium.
3. BS sequencing analysis revealed the presence of the two methylation motifs in the upstream regions of about 2150 genes overall genome.
4. Two methylation motifs could have a role in regulation the gene expression.

References

1. Kahramanoglou *et al.* (2012) Nat Commun. e 3:886.
2. Fukuda *et al.* (2008) Genome Biol. 9:R163.
3. Yagüe *et al.* (2013) FEMS Microbiol Lett. 342:79-88.
4. Novella IS & Sánchez J. (1995) Res Microbiol., 146:721-8.

Acknowledgements

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